Initial resuscitation with plasma and other blood components reduced bleeding compared to hetastarch in anesthetized swine with uncontrolled splenic hemorrhage

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BACKGROUND: Damage control resuscitation recommends use of more plasma and less crystalloid as initial resuscitation in treating hemorrhage. The purpose of this study was to evaluate resuscitation with either blood components or conventional fluids on coagulation and blood loss.

STUDY DESIGN AND METHODS: Isofluorane-anesthetized, instrumented pigs (eight per group) underwent controlled hemorrhage of 24 mL/kg, 20-minute shock period, splenic injury with 15-minute initial bleeding, and hypotensive fluid resuscitation. Lactated Ringer's (LR) was infused at 45 mL/kg while hetastarch (high-molecular-weight hydroxyethyl starch 6%, Hextend, Hospira, Inc., Lake Forest, IL) and blood component (fresh-frozen plasma [FFP], 1:1 FFP:[red blood cells] RBCs, 1:4 FFP : RBCs, and fresh whole blood [FWB]) were infused at 15 mL/kg. Postresuscitation blood loss (PRBL), hemodynamics, coagulation, hematocrit, and oxygen metabolism were measured postinjury for 5 hours.

RESULTS: Resuscitation with any blood component reduced PRBL of 52% to 70% compared to Hextend, with FFP resulting in the lowest PRBL. PRBL with LR (11.5 ± 3.0 mL/kg) was not significantly different from Hextend (17.9 ± 2.5 mL/kg) or blood components (range, 5.5 ± 1.5 to 8.6 ± 2.6 mL/kg). The volume expansion effect of LR was transient. All fluids produced similar changes in hemodynamics, oxygen delivery, and demand despite the oxygen-carrying capacity of RBC-containing fluids. Compared with other fluids, Hextend produced greater hemodilution and reduced coagulation measures, which could be caused by an indirect dilutional effect or a direct hypocoagulable effect.

CONCLUSIONS: These data suggest that blood products as initial resuscitation fluids reduced PRBL from a noncompressible injury compared to Hextend, preserved coagulation, and provided sustained volume expansion. There were no differences on PRBL among RBCs-to-FFP, FWB, or FFP in this nonmassive transfusion model.

Hemorrhage remains a leading cause of death from severe traumatic injuries in both the civilian and the military environments, up to 40% of civilian and 50% of military deaths.[1-3] Injuries to the legs and arms are easily accessed and treated with advanced bandages[4,5] and tourniquets.[6] What to do in the prehospital and preoperative care of injured patients with uncontrolled truncal hemorrhage is an area of active research.[7]

In the emergency and operating rooms, damage control resuscitation strategies,[8,9] composed of permissive hypotension, reduced crystalloid use, and increased plasma-to-red blood cell (RBC) ratios, has been associated with reduced mortality in the severely injured trauma patients requiring massive transfusion in both civilian[10] and military casualties,[11-13] although this association is not

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 APR 2011	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Initial resuscitation with plasma and other blood components reduced bleeding compared to hetastarch in anesthetized swine with uncontrolled splenic hemorrhage	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
Sondeen J. L., Prince M. D., Kheirabadi B. S., Wade C. E., Polykratis I. A., de Guzman R., Dubick M. A.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX	

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**UU**	**14**	

without controversy.[14,15] The rationale behind reducing crystalloid use and utilizing plasma early in treatment is to prevent dilution of remaining coagulation factors or reverse the coagulopathy that has been observed in severely injured trauma patients due to consumption of factors or other unknown mechanisms.[16] Although some studies have shown a reduction in the amount of blood products that were administered presumably because of less bleeding when using damage control resuscitation techniques,[10] a direct effect of plasma administration to act as a hemostatic agent to reduce bleeding remains to be established, particularly with prehospital resuscitation.[17]

In austere environments such as the remote battlefield or in circumstances of mass civilian casualties, the principles of damage control and hemostatic resuscitation strategies could be applied to the initial prehospital care of casualties with potentially survivable, severe noncompressible hemorrhage. The retrospective studies mentioned above compared various blood product ratios to other ratios and not to a control group utilizing a crystalloid or colloid fluid, so their efficacy as a hemostatic agent cannot be directly accessed. This study was designed to compare blood products with the standard of care (hetastarch (high-molecular-weight hydroxyethyl starch 6%, Hextend, Abbott Laboratories, Inc., North Chicago, IL and lactated Ringer's [LR] solution), in conjunction with permissive hypotension, as an initial resuscitation fluid in an uncontrolled swine hemorrhage model (noncoagulopathic) and evaluated each fluid's ability to reduce bleeding, maintain coagulation function, and restore hemodynamics. In addition, we investigated whether limiting the resuscitation volume to an amount of blood products, which could potentially be carried far forward would preserve the physiological and coagulation status under a scenario of delayed definitive care.

MATERIALS AND METHODS

This study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research (Fort Sam Houston, TX) and performed in compliance with the Animal Welfare Act and in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 1996).

General procedures

Yorkshire-cross female pigs weighing 38.7 ± 0.3 kg were obtained from Midwest Swine Research (Gibbon, MN). They were held a minimum of 72 hours after receipt for acclimation and stabilization. After this period, baseline screening hematologic samples (complete blood count, blood chemistries, and coagulation tests) were obtained and daily health assessments made during the week before the study. All animals were fed laboratory-grade commercial feed. Water was provided ad libitum to all animals via an automated water delivery system (Lixits, Lixit Corp., Napa, CA).

Surgical instrumentation

The pigs were fasted 12 to 18 hours before surgery with water available ad libitum. Before surgery, the pigs were injected with glycopyrrolate (Robinul, 0.01 mg/kg, Baxter Healthcare, Deerfield, IL) and tiletamine-zolazepam (Telazol, 8 mg/kg, Wyeth, Fort Dodge, IA) intramuscularly, for saliva secretion control and sedation, respectively. Anesthesia was induced via a facemask with approximately 5% isoflurane (Forane, Baxter Healthcare) in 100% oxygen. The animals were then intubated with a cuffed endotracheal tube (7.5 mm, Rusch, Teleflex Medical, Research Triangle Park, NC). During surgical instrumentation, anesthesia was maintained with 1% to 3% isofluorane in 30% oxygen in air using a ventilator and monitor (Fabius gas anesthesia system and Infinity Explorer monitoring system, Draeger Medical, Telford, PA). A critical care monitor (S/5 Datex-Omeda, GE Healthcare, Waukesha, WI) was attached to the endotracheal tube for continuous noninvasive measurement of oxygen consumption. Animals were placed in the supine position; the ventral cervical area, ventral abdomen, and left femoral area were clipped; and an electrocardiogram (ECG) monitor (Draeger Medical) for measuring heart rate was secured and continuous monitoring started. Tidal volume was initially set at 7 mL/kg with a rate of 25 breaths/minute. Ventilation was adjusted to maintain an end tidal pCO_2 of approximately 40 mmHg. Core temperature was monitored with an esophageal thermometer (Draeger Medical) and maintained between 37 and 39°C using a water-filled blanket (Medi-Therm II hyper-/hypothermia system, Gaymar Orchard Park, NY) and a forced-air warming system (Bair Hugger, Augustine Medical, Inc., Eden Prairie, MN). Urine was collected via a Foley catheter (10 Fr., 3-mL balloon, all silicone, Sherwood Medical, St Louis, MO), placed transuretherally, and measured with a closed-system urometer (Professional Medical Products, Greenwood, SC).

Vascular catheters were inserted via cut downs. A pressure transducer-tipped catheter (Mikro-Tip, Millar Instruments, Inc., Houston, TX) was placed nonocclusively into the carotid artery for blood pressure monitoring. A Swan-Ganz catheter was inserted into the pulmonary artery through the left jugular vein for continuous measurement of cardiac output and central venous pressure (Opti-Qvue CCO System, Hospira, Inc.). A catheter (0.050 in., Tygon polyvinyl chloride, Cole Parmer, Inc, Vernon Hills, IL) was placed occlusively into the same

jugular vein for blood sampling and infusion of calcium chloride (25 mL of a 4% solution [1 g], Hospira, Inc.) during blood product administration or equal volume of saline for nonblood fluids. Other catheters were placed occlusively in the left femoral artery and vein (8 Fr. side-port/percutaneous catheter introducer, Argon Medical Devices, Athens, TX) for arterial hemorrhage, blood sampling, and intravenous (IV) infusion of the resuscitation fluid. A laparotomy was performed to allow access to the spleen. After suctioning existing fluid, a sheet of plastic (emptied saline bag cut so that it lies flat) was placed between the spleen and the intestines. Suction tubes (Via-Guard, SurgiMark, Inc., Yakima, WA) with perforated tips were placed in the peritoneal cavity in such a way that the blood from the injured spleen could be collected. The animals were not heparinized; the catheters were kept patent by a slow continuous infusion of nonheparinized saline through an intraflow device (3 mL/hr, Intraflow continuous flush devices, Abbott Laboratories, Abbott Park, IL), which were attached to bags of normal saline pressurized to 300 mmHg using a Level 1 blood warmer (Smiths Industries Medical Systems, Rockland, MA).

Experimental procedure

After the instrumentation was completed and the mean arterial blood pressure stabilized, a 10-minute baseline period began and hemodynamic measurements were made. All data from the analog and RS-232 signals were collected on a data acquisition instrumentation rack/ biomedical data recorder and physiological data recorder program (Dynamic Research Evaluation Workstation—DREW, US Army Institute of Surgical Research, San Antonio, TX). A baseline arterial sample (27 mL) was drawn for complete blood count (2 mL; Cell-Dyn 3700CS hematology analyzer, Abbott Laboratories), coagulation tests (4.5 mL, BCS, Dade Behring, Deerfield, IL) including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, and thromboelastography (TEG, Haemoscope 5000, Haemonetics Corp., Braintree, MA), as well as total protein (5 mL, Dimension Xpand chemistry analyzer, Dade Behring), and arterial and venous blood gas (ABG; 2×3 mL, COBAS b221 blood analyzer system, Roche Diagnostics, Basel, Switzerland) determinations. Blood samples for TEG were collected in 4.5-mL citrate tubes and allowed to equilibrate for 15 minutes at room temperature before measurements. A 1-mL aliquot was then pipetted into a kaolin tube to initiate coagulation and 340-μL samples were pipetted into TEG cups with 20 μL of calcium chloride (0.2 mmol/L). The clotting process was traced at the animal's core temperature (approx. 39°C) by the TEG machine.

Next, a controlled hemorrhage was performed by removing 24 mL of blood/kg at 100 mL/min by using our custom servocontrolled computerized pump program (LabView, National Instruments, Inc., Austin, TX), as previously described.[18] Blood was collected into sterile blood bank bags (three-bag collection set, citrate-phosphate-dextrose-adenine [CPDA] and AS-5, Terumo Products, Somerset, NJ). A pump with computerized drive (Masterflex, Cole-Parmer Instrument Co., Vernon Hills, IL) was used to withdraw the blood from the femoral artery catheter and collected in the blood bags, which were placed on a scale (SR16000 Mettler Balance, Mettler-Toledo, Greifensee, Switzerland). No more than 450 g (specific gravity of pig blood is 1.04 g/mL) of blood was collected into each blood bag until a total of 24 mL of blood/kg was obtained. If the mean arterial pressure (MAP) decreased to 15 mmHg during the controlled hemorrhage, the pump was stopped until the pressure rose above 20 mmHg. Also, if the MAP dropped below 30 mmHg at any time during the study period, the isofluorane was temporarily turned off to allow the MAP to increase and prevent unexpected death since shocked animals are very sensitive to anesthetic agents. Animals were closely monitored to determine needed adjustments of the isofluorane dosage required to maintain a surgical plane of anesthesia. The collected blood was processed (described below) for RBCs and fresh-frozen plasma (FFP), which were used for subsequent experiments, thus reducing or eliminating the need for donor animals. The blood was type-matched from the screening samples (pigs have A or O blood type, Eldon home kit 2511-1, Eldon Biologicals, A/S, Gentofte, Denmark) and type-specific FFP and RBCs were used for resuscitation.

After the controlled hemorrhage, the splenic injury was made. Using a skin marker, a line was drawn down the entire length of the spleen 1 cm lateral to the midline to avoid injuries to large arteries and veins in the spleen. The spleen was then cut through and through with a No. 21 scalpel blade along the drawn line. The uncontrolled hemorrhage volume from the splenic injury was measured continuously by suctioning shed blood into canisters (Vac Rite disposable suction system, Baxter Healthcare), which had been placed on a balance (SR16000 Mettler Balance, Mettler-Toledo). Suction tubing with perforated sleeves was placed in the abdomen so as not to influence the blood loss from the spleen injury. The balance was connected to a computer and the weight was continuously recorded. A third hand-held suction tube was used to collect blood from the surface of the spleen, without disturbing the clots. The time that the splenic injury was completed was designated as the zero time point. At 15 minutes, hemorrhage volume was measured, and blood samples were collected for laboratory measurements. Preliminary studies indicated that the majority of the splenic bleeding, except for some oozing, is complete by 15 minutes in this model. This time was also selected as a time when a combat medic would be expected to be able to begin treatment of an injured casualty on the battlefield.

Resuscitation was started with the appropriate fluid using our servocontrolled computerized pump LabView-based program. Forty-eight pigs were randomly distributed among six groups (n = 8/group) and resuscitated with LR solution, Hextend, FFP, 1:1 FFP : RBCs, 1:4 FFP : RBCs, or fresh whole blood (FWB). When the total volume of each fluid was administered, no additional fluid was given. Fifteen mL of blood product or colloid/kg was given at 1 mL/kg/min and 45 mL of crystalloid/kg was infused at 1.5 mL/kg/min because of viscosity differences. Animals were monitored for 5 hours after the injury or until death, during which time hemodynamic and coagulation data were collected. In addition to baseline and pre-resuscitation (15 min postinjury) samples, additional blood samples were collected at 30, 60, 90, 120, 180, 240, and 300 minutes after splenic injury or at death.

At various time points (baseline; 30, 60, and 300 minutes; or death), jejunum tissue samples were collected for wet or dry weight (edema) determinations. For serial collection of the samples, four circular areas 1 cm in diameter were marked by placing purse-string sutures (2-0 Vicryl with an atraumatic needle) in nonvascularized areas of the jejunum. The first site was made approximately 100 cm from the ileocecal junction and the subsequent sites were about 10 cm distal to the initial site. To collect the sample, we exposed the appropriate site on the jejunum, tightened and tied the purse-string suture, and biopsied the exteriorized tissue inside the purse string ensuring that there was no bleeding or leakage of intestinal contents into the peritoneal cavity. Each biopsy was cut into three pieces placed into aluminum pockets, weighed (wet weights, 20 to 50 mg, Mettler AT261 delta range scale, Mettler-Toledo), and dried at 100°C temperature in an oven (Model SW-11TA-1, Blue M, a Unit of General Signal, Blue Island, IL) for approximately 3 days until the tissues were totally dry and the weight was stable. The data were expressed as the percentage of water with the formula:

$$\% \text{ water} = 100 \times \left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \right)$$

At the end of 5 hours or at death time (taken to be when end-tidal $pCO_2 \leq 15$ mmHg or flat-line ECG), after collection of the final blood and tissue samples, each pig was humanely euthanized with sodium pentobarbital (90 mg/kg IV, 10 mL Fatal Plus, Vortech Pharmaceuticals, LTD, Dearborn, MI) while still under surgical anesthesia.

Nonresuscitated group from a preliminary study

In a pilot study, we used the same methods described for the study animals but there was no resuscitation fluid administered. The data from a preliminary model development study (n = 3) of nonresuscitated pigs are shown in

the first two figures for elucidation of this new model, but were not included in any statistical evaluation.

Donor pigs

The blood collected during the controlled hemorrhage was used to prepare RBCs and FFP for blood component therapy to minimize the number of donor pigs required for other experiments. For the FWB and 1:4 ratio groups, the donor pigs (n = 16) were anesthetized just as the other pigs and ECG leads placed. A carotid artery was cannulated via a cutdown and blood was collected rapidly into blood transfusion bags containing standard CPDA anticoagulant (three-bag collection set, CPDA and Optisol AS-5, Terumo Products) using a Masterflex pump, as described for the controlled hemorrhage procedure. After blood collection, the animals were euthanized with sodium pentobarbital (90 mg/kg IV, 10 mL). The blood was either separated into its components: the RBCs were stored overnight at 4°C and used the next day; the FFP was frozen at –20°C and thawed in the morning of the study in a 37°C water bath or used as FWB on the same day of collection.

Blood component separation technique

The bags containing blood were centrifuged ($5000 \times g$, Model RC-3B with H6000A rotor, Dupont Sorvall Instruments, Claremont, CA) at room temperature for 5 minutes after reaching speed with brake set on low (Setting 2). Plasma was then extracted with a plasma extractor (Model 4R4414, Fenwal, Inc., Lake Zurich, IL) into a satellite bag, the tubing was stripped and sealed with clips (hand stripper, No. R4453, Fenwal; hand sealer, No. 4R4454, Fenwal; clips, No. 4R4418, Fenwal) and frozen at –20°C. A blood preservative solution (100 mL Optisol, Terumo) was added to the RBCs and the mixture was stored in a refrigerator overnight at 4°C (HLT-5V-4BBABA blood bank refrigerator, Harris, Asheville, NC).

International normalized ratio estimation and TEG-G calculation

We estimated the international normalized ratio (INR) by taking the average of the baseline PT measurements of all the pigs as our "normal" population PT for use as the denominator. We then calculated the INR by dividing the PT at each time point by the "normal" PT.

The maximum amplitude (MA; mm) of the TEG was converted into the shear elastic modulus strength (dyn/cm^2), designated TEG-G, by the formula

$$\text{TEG-G} = \frac{5000 \times (\text{TEG} - \text{MA})}{(100 - (\text{TEG} - \text{MA}))}$$

(automatically calculated by the software).

Oxygen metabolism calculations

Oxygen delivery (DO_2; mL O_2/kg/min) was calculated as

$$DO_2 = \frac{CaO_2 \times \text{Cardiac Output}}{\text{body weight}},$$

where CaO_2 is the arterial oxygen content measured in (mL O_2/100 mL of blood, the cardiac output is measured in mL/min, and the body weight is in kg. Oxygen demand (mL O_2/kg/min) was calculated as

$$O_2 \text{ demand} = (\text{Plasma lactate}) + VO_2,$$

where the lactate concentration was measured in mmol/L in the arterial blood gas sample and VO_2 is the oxygen consumption value (mL O_2/min, measured with the S/5 critical care monitor using the assumptions as per Hannon and colleagues.[19] The DO_2-to-oxygen demand ratio was calculated by division. Oxygen extraction ratio (OER) was calculated by OER = 100 × [(arterial oxygen content – venous oxygen content) / arterial oxygen content].

Statistical analysis

Data are expressed as mean ± SEM. This study was powered to be able to detect 50% reduction in postresuscitation blood loss (PRBL) with an alpha level of 0.05 and power of 0.80. Data analyses were carried out by using computer software (Statistical Analysis System package, SAS, Cary, NC). The lifetest procedure log-rank test was used to evaluate censored survival time data. The data for body weight, controlled hemorrhage volume, initial blood loss after spleen injury, PRBL, and resuscitation volume were analyzed by analysis of variance (ANOVA; generalized linear mode procedure) followed by Student-Newman-Keuls test for post hoc comparisons. Variables measured at different times (hemodynamic values, all data from the blood samples, oxygen metabolism data and ratios, percentage of tissue water) were analyzed by using a two-way ANOVA (mixed procedure) allowing for factors of treatment, time (repeated measures), and treatment-by-time interaction. If the treatment-by-time interaction was significant, individual comparisons of the different treatments were made as follows: If variances were equal among treatment groups (Levene's test) of the data, one-way ANOVA was used to test the treatment effect at each time of measurement for each variable, followed by Tukey's method for post hoc comparisons. If the variances were not equal, the Kruskal-Wallis nonparametric test was used, followed by Bonferroni correction for post hoc comparisons. If the time factor was significant in the two-way ANOVA analysis, each group was analyzed using a one-way ANOVA with Dunnett's post hoc test comparing each time with the baseline value if variances were equal among treatment groups (Levene's test) of the data. If the variances were not equal, the Kruskal-Wallis nonparametric test was used, followed by Bonferroni correction for post hoc comparisons of each time with the baseline value. Significance was determined as a p value of less than 0.05.

RESULTS

Blood loss, resuscitation volumes, and survival

The PRBL in the blood component groups was significantly lower (FFP, 70 ± 8%; 1:1 FFP : RBCs, 55 ± 14%; 1:4 FFP : RBCs, 58 ± 13%; and FWB, 52 ± 15%) than the Hextend group (see Fig. 1). The blood loss in the LR group was 36 ± 17% lower than Hextend, but this was not significant. There was a range of percentage survival at 5 hours (see Fig. 2) between 25% survival in the LR and Hextend groups to 50% or 62.5% survival in the groups that received blood products, but there were no significant differences possibly due to limited power. In model development experiments with no fluid resuscitation (n = 3), the comparable injury resulted in 100% death within 2 hours after splenic injury (range, 39-117 min).

As indicated, all groups were subjected to a fixed controlled hemorrhage of 24.1 ± 0.01 mL/kg or an estimated 34% of the animal's estimated blood volume (70 mL/kg [personal observations based on ICG plasma volume determinations on pigs of this weight]). The blood loss that occurred during the first 15 minutes after the spleen injury was 7.0 ± 0.4 mL/kg for all animals. The only difference in blood loss among the groups was solely due to the blood loss after fluid resuscitation.
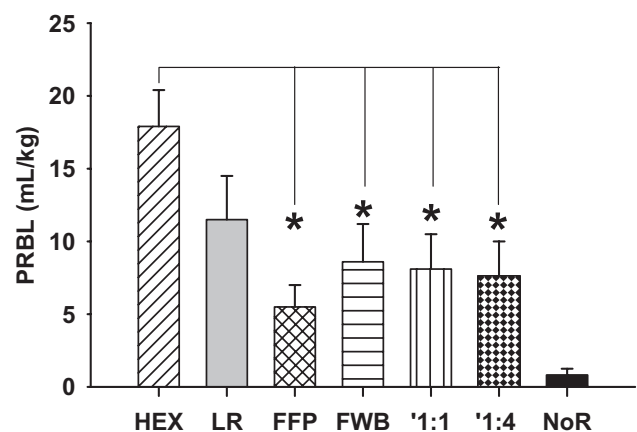

Fig. 1. PRBL(s) from the spleen injury in the six treatment groups FFP, 1:1 and 1:4 FFP : RBCs, and FWB groups were significantly different from the Hextend group. The nonresuscitated control group data are shown for comparison, but was not statistically evaluated. HEX = Hextend; 1:1 = 1:1 ratio of FFP : RBCs; 1:4 = 1:4 ratio of FFP : RBCs; NoR = no resuscitation. *p < 0.05 different from Hextend.
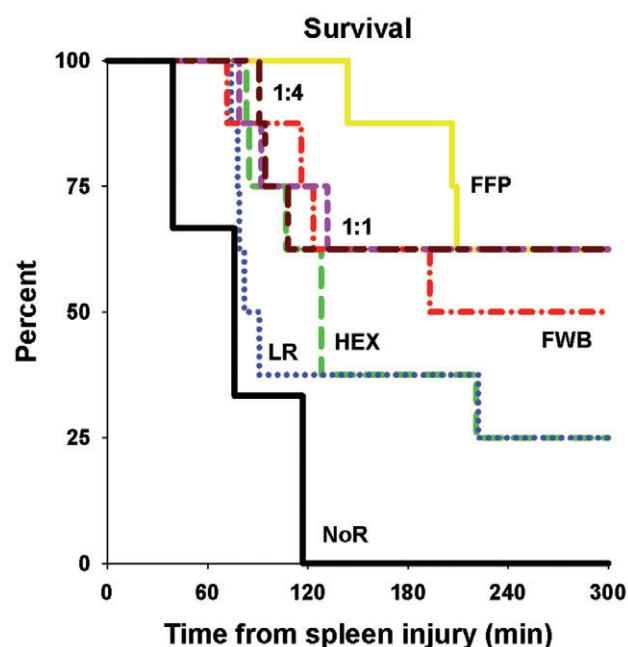
Fig. 2. Kaplan-Meier survival graph. The abbreviations for the groups are the same as given in the legend for Figure 1. There were no significant differences. The nonresuscitated control group data are shown for comparison, but were not statistically evaluated. There were n = 8 per group for all the groups except for n = 3 for the no-resuscitation group.

Fig. 3. Standard coagulation variables of PT, aPTT, and fibrinogen concentration. The letters next to the symbols denote which groups are significantly different from those values at each time point. L = LR; F = FFP; 1 = 1:1 ratio; 4 = 1:4 ratio; B = FWB. The abbreviations for the groups are the same as given in the legend for Figure 1. The dashed lines denote the minimum and maximum values of normal swine samples from our laboratory. For each time point, the means of the values are for those animals that survived (see Figure 2 for survival data). For the death sample, the number of animals for each group were: Hextend = 6; LR = 6; FWB = 4; FFP = 3; 1:1 = 3; 1:4 = 3.

Coagulation status

Groups receiving blood products maintained their PT, aPTT, and fibrinogen levels throughout the study, whereas Hextend treatment resulted in a significant prolongation of the PT at 60 minutes or later, a prolongation of the aPTT at 60 minutes, and a reduction in the fibrinogen concentration at 30 and 60 minutes (Fig. 3). The effect on PT with the LR treatment was significantly different from the FFP group only at 30 minutes; it then returned toward baseline levels. The changes that are significantly different from baseline (not depicted on the graph for clarity) are as follows:

- For PT, only the LR (30 min) and Hextend (60 and 180 min) groups were significantly elevated from baseline.
- For fibrinogen concentration, only Hextend (30 min) was significantly reduced from baseline.

The maximum calculated INR at 60 minutes for the LR group was 1.1 ± 0.02, for Hextend it was 1.2 ± 0.07, and for all the other blood product groups it remained at 1.0 ± 0.02. With regard to TEG measurements, only the Hextend group elicited a significant reduction compared to the other treatments (specified in Fig. 4) at the 30- and 60-minute time points in the K, angle, MA, and shear
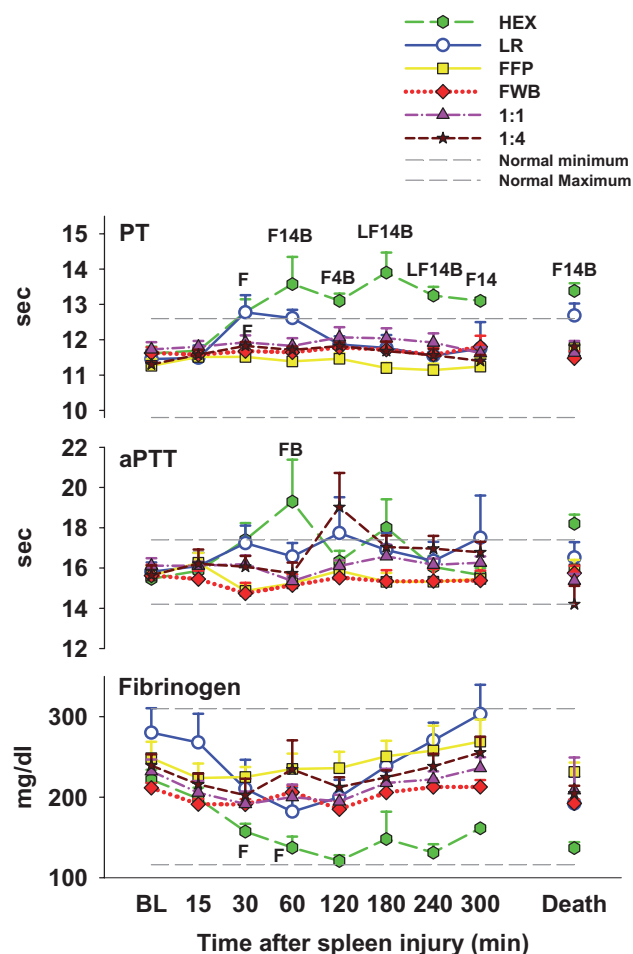
elastic modulus (G) indicating a hypocoagulable state, but not in the R variable. No other groups showed altered TEG variables in response to the hemorrhage or resuscitation. The changes that are significantly different from baseline (time course changes not depicted on the graph for clarity) are as follows:

- For TEG-R, the values at 15, 30, and 60 minutes for FFP and 1:1 FFP : RBCs were reduced from baseline.
- For TEG-K, the value at 60 minutes for Hextend was increased from baseline.
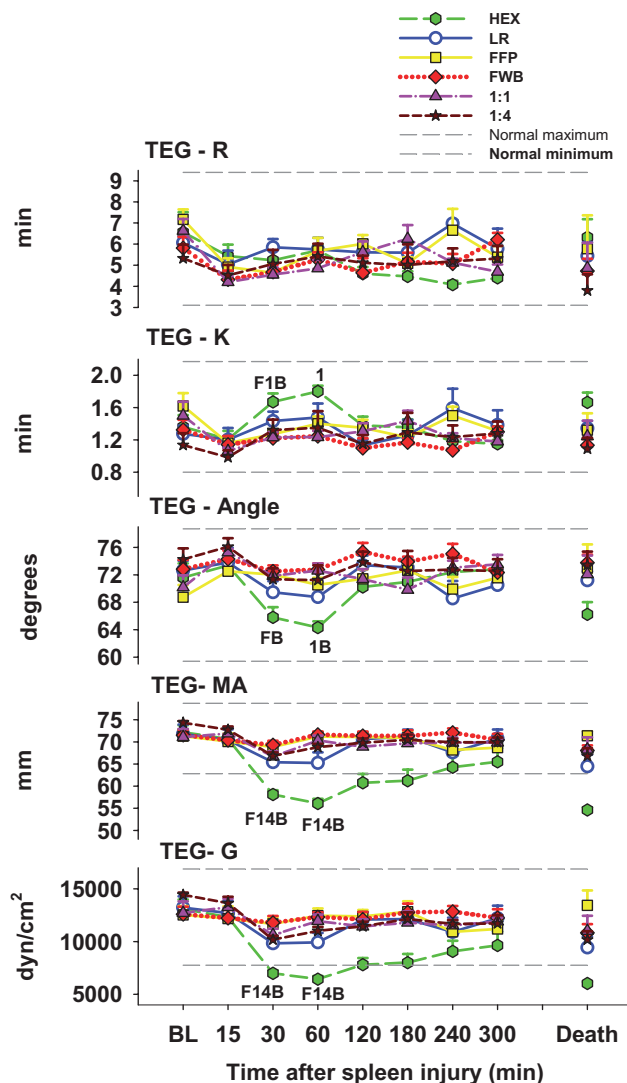
Fig. 4. TEG variables of R (clot initiation time), K (clotting time to a fixed 20-mm of clot strength), angle (kinetics of clot development), MA (maximum amplitude, maximum clot strength), and G (shear elastic modulus). The letters below the symbol(s) denote which groups are significantly different from those values at each time point. L = LR; F = FFP; 1 = 1:1 ratio; 4 = 1:4 ratio; B = FWB. The abbreviations for the groups and description of the dashed lines are the same as given in the legend for Figure 3. BL = baseline.

- For TEG-angle, no significant changes occurred with time with any treatment.
- For TEG-MA, the levels were reduced from baseline for Hextend (30 and 60 min).
- For TEG-G, the levels were reduced from baseline for Hextend (30 and 60 min).

For the coagulation variables presented in this article, normal values for pigs in our laboratory are listed in Table 1. These values come from the baseline samples of instrumented pigs from previous studies in our laboratory.[20,21] The normal range is plotted in Figs. 3 through 5 to illustrate the relationship between the values after severe hemorrhage and limited resuscitation with the values that are considered to be normal baseline values. These normal values found for anesthetized, instrumented pigs compares well with the expected range for normal humans, although the minimum for TEG-angle and TEG-MA are lower than in our pigs. This indicates that pigs may be slightly more coagulable than humans, but not outside of normal human ranges. A recent study found that human TEG values for a normal population had a wider range than those reported by the manufacturer, and our pig ranges fall within this published range.[22]

Hemodilution and tissue edema status

As expected, resuscitation with FFP, LR, and Hextend produced a significant reduction in hematocrit (Hct) and hemoglobin (Hb) concentration compared to any of the groups that received RBCs (Fig. 5). Despite receiving the identical 15 mL/kg volume as the FFP group, the Hct and Hb concentration in the Hextend group was significantly lower than in the FFP and LR groups after resuscitation. In contrast, the platelet (PLT) count did not differ significantly among the groups except at the 60-minute time, when the PLT count in the FWB group was higher than that in the Hextend group.

Despite the effect of diluting the RBCs, the plasma protein concentration was maintained higher in the FFP, 1:1, 1:4, and FWB groups than in the LR and Hextend groups. There was a transient dilution of the plasma protein concentration with LR resuscitation at 30 and 60 minutes, with an increase after 60 minutes, most likely due to the LR distributing throughout the extracellular fluid space. Similar to the effect on the Hct and Hb concentration, the plasma protein concentration was significantly ($p < 0.05$) lower in the Hextend group than all other groups at 60 minutes, and it appeared to remain low for the entire period of observation, although it did not reach significance at other time points.

The changes in Fig. 5 that are significantly different from baseline (not depicted on the graph for clarity) are as follows:

- For Hct, the LR (30, 60, and 120 min), Hextend (all time points after 15 min), and FFP (all time points after 15 min) groups were reduced, and the 1:4 FFP : RBCs group (all time points after 15 min) was increased from baseline.
- For Hb concentration, the LR (60 min), Hextend (30 and 60 min), and FFP (30, 60, 130, and 180 min) groups were reduced; the 1:4 FFP : RBCs (all time points after 15 min) group was increased from baseline.

TABLE 1. Normal coagulation values for pigs and humans for our laboratory*						
	Species					
	Pig				Human	
Variable	Number	Mean ± SD	Minimum	Maximum	Minimum	Maximum
PT (sec)	130	11.3 ± 0.6	9.9	12.6	7.8	9.4
PTT (sec)	122	15.8 ± 0.7	14.2	17.4	22.8	31
Fibrinogen (mg/dL)	125	205 ± 40	116	310	180	350
PLT count (×10⁹/l)	130	372 ± 79	194	541	148	352
TEG-R (min)	126	6.4 ± 1.4	3.1	9.4	3	8
TEG-K (min)	129	1.4 ± 0.3	0.8	2.2	1	3
TEG-angle (°)	126	70.1 ± 4.3	59.4	78.7	55	78
TEG-MA (mm)	126	70.9 ± 3.8	62.8	78.7	51	69

* For human ranges: PT, PTT, and fibrinogen ranges from Dade Behring. PLT count from Abbott Laboratories. Citrated Kaolin TEG values from Haemoscope.

- For PLTs, the LR (30 and 60 min), Hextend (30 and 60 min), 1:1 FFP : RBCs (all time points after 15 min), and 1:4 FFP : RBCs (all time points after 15 min) groups were reduced from baseline.
- For total protein concentration, the LR (30 and 60 min), Hextend (30 and 60 min), FFP (30, 60, 120, 240, and 300 min), 1:1 FFP : RBCs (30 min), 1:4 FFP : RBCs (30, 60, 120, 180, and 240 min), and FWB (30 min) groups were reduced from baseline.

The distribution of the LR to the extravascular space is confirmed by the significant increase in percentage of water at 60 minutes found in the serial samples of the jejunum (Fig. 6) compared to the Hextend group. This 60-minute value was also significantly elevated from the baseline value.

Hemodynamic, acid-base status, metabolic, and organ function data

Despite the different effects on the plasma protein, coagulation variables, and cellular components of the blood, all treatments resulted in similar changes in MAP and central venous pressure, heart rate, and total peripheral resistance (only MAP data are shown). Thus, for simplicity, the changes in MAP were averaged over all animals in the six groups and presented in Fig. 7. The controlled hemorrhage resulted in a low MAP of 28 ± 1 mmHg. There was a spontaneous increase in MAP after the controlled hemorrhage, such that the MAP at 15 minutes was higher than that measured immediately after the controlled hemorrhage. Resuscitation raised the MAP and central venous pressure, but not to baseline levels. To ensure that a hypotensive level of resuscitation was maintained, the resuscitation pump was to be turned off when MAP reached 65 mmHg. However, in only one animal of the entire study was that target met; in all other animals, resuscitation was infused continuously. During and after resuscitation, MAP was approximately 50 mmHg as seen in Fig. 7.

The cardiac output (Fig. 7) for the Hextend group tended to be elevated compared to the other groups, but it was not significant. The cardiac output was less than the baseline value at the end of the controlled hemorrhage and the 15-minute time point in all groups, but resuscitation with Hextend returned the cardiac output to baseline values. In contrast, the cardiac output in all of the other treatment groups remained below the baseline values throughout the observation period (Fig. 7). All of the resuscitation fluid was administered within the first 60 minutes. There were small changes in the arterial blood gases (pO₂, pCO₂, pH, base excess) in response to the hemorrhage but all values returned to baseline levels after resuscitation (data not shown).

Although three of the groups received oxygen-carrying RBCs, there were no significant differences in the ability of any of these fluids to maintain DO_2 or its relationship to oxygen demand; thus, all the data were combined in Fig. 8. This hemorrhagic shock model resulted in an initial reduction in DO_2 to 37 ± 2% of baseline. Resuscitation brought it up to 55 ± 1% of baseline values. Despite a significant reduction in Hb concentration in the Hextend group (Fig. 5), the trend for the increase in cardiac output (Fig. 7) compensated for this deficit, resulting in a comparable DO_2 in all groups. There was no significant change in oxygen consumption (data not shown). This level of hemorrhagic shock led to a maximum of 152 ± 5% increase in oxygen demand above baseline at 30 minutes, and resuscitation returned it to a level not different from baseline. At baseline conditions, the DO_2 to demand ratio was 2.1 ± 0.5. Hemorrhagic shock reduced the ratio to 0.70 ± 0.04 at 15 minutes, and resuscitation increased the ratio to a maximum of 0.97 ± 0.07 at 240 minutes. The fact that this ratio remained below 1 even with fluid resuscitation was consistent with the high mortality rate in this model. Another indication of the incomplete resuscitation brought about by the small volume of resuscitation is that the oxygen extraction ratio remained elevated above 60% for the duration of the experiment.
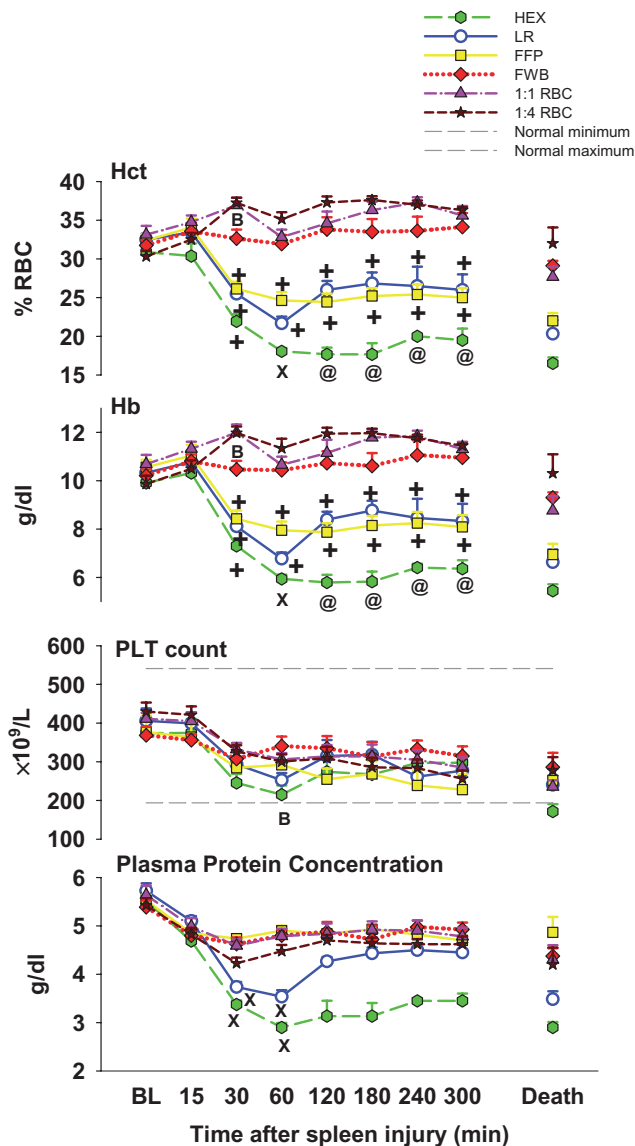
Fig. 5. Hct and Hb concentrations, PLT count, and total plasma protein concentration. The letter B denotes that the group is significantly different from the FWB group at that time point. The plus (+) sign next to the symbols in the graph denote that those groups are significantly different from 1:1, 1:4, and FWB groups at those time points. The letter X next to symbols denotes that the group at that time point is significantly different from the FFP, 1:1, 1:4, and FWB groups. The ampersand (@) denotes that the Hextend group is significantly different from all other groups at those time points. The dashed lines for PLT count panel denote the minimum and maximum values of normal swine samples from our laboratory.

There were slow increases in plasma creatinine (from 1.2 ± 0.2 to 2.7 ± 0.5 mg/dL) and potassium (from 4.0 ± 0.04 to 6.5 ± 0.18 mmol/L; Fig. 8) levels throughout the course of the study, suggesting that kidney function was compromised with this partial resuscitation. The urine flow rate was 0.69 ± 0.05 mL/min during baseline and fell to 0.15 ± 0.02 mL/min over the course of the 5-hour experiment. Although not significant, there were

trends toward increased aspartase aminotransferase (AST) and creatine kinase values as well (data not shown).

DISCUSSION

The results of our hypotensive resuscitation study confirmed that the use of a blood product as an initial resuscitation fluid acted as a hemostatic agent and reduced PRBL by more than half compared to Hextend. LR resuscitation resulted in a hemorrhage volume in between those of blood products and Hextend. The fluid with the highest amount of coagulation factors, FFP resulted in the lowest blood loss. The FFP treatment had the highest amount of coagulation proteins since the plasma was not diluted with RBCs as the total volume of each blood product was the same, 15 mL/kg. There were no significant differences in PRBL among any of the blood products, including FFP, component therapy with 1:1 or 1:4 ratios of FFP : RBCs, which did not contain PLTs, and FWB, which did contain PLTs. The fact that there was no thrombocytopenia in this model may explain why added PLTs, equivalent to that in 1 unit of whole blood–derived PLTs, did not affect the results.[23]

The finding that there were no differences between the 1:1 and 1:4 ratios is probably a reflection of the design of this model: although a lethal model, it was not a replicate of massive transfusion therapy. This model may be relevant to the 23.4% of 23,250 injured casualties over 5 years in Iraq who required limited blood transfusion, compared to 6.4% who needed a massive transfusion.[24] There was no additional fluid administration in this model during 5 hours of observation that mimicked prolonged evacuation. The Basic Management Plan for Tactical Field Care[25] recommends the administration of Hextend (up to 1 L) for initial fluid resuscitation of combat casualties in a shock state on the battlefield. Preliminary information in the Ranger Prehospital Trauma Registry detailing the treatment of 419 casualties wounded on the battlefield revealed that 13% received fluid in the tactical environment. Of those who received fluid, 36% were treated with Hextend, while the rest (64%) were resusci-
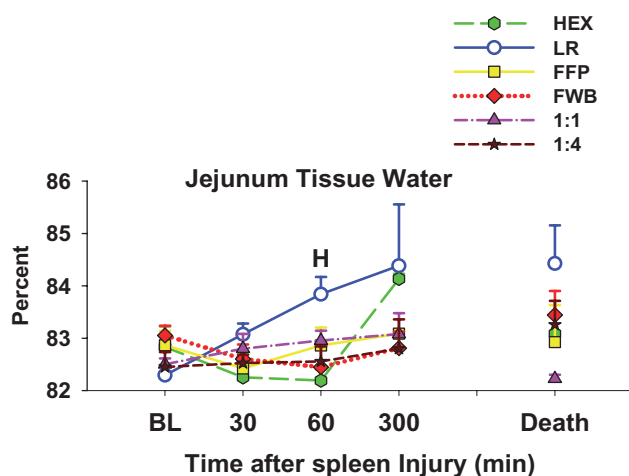
Fig. 6. The wet to dry weight ratio expressed as percentage of water of serial biopsies of the jejunum. H denotes that there is a significant difference from the Hextend group at that time point.
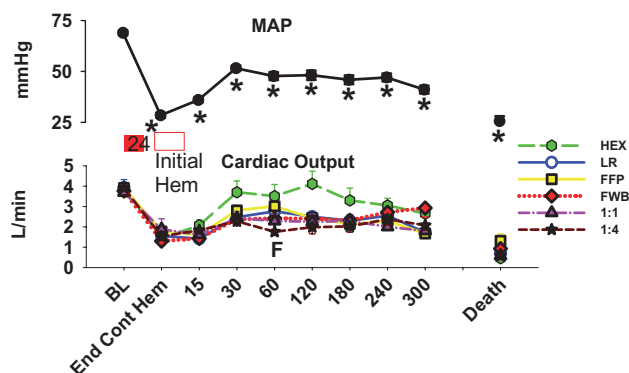

Fig. 7. The time course of the hemodynamic data. There were no differences between the groups for the MAP, so the mean values for all animals are shown for simplicity. The asterisks denote when the values were significantly different from baseline values. The cardiac output data are shown for the individual groups, where F = FFP difference at 60 minutes for the 1:4 FFP : RBCs group. BL = baseline; EndContHem = end of the controlled hemorrhage.
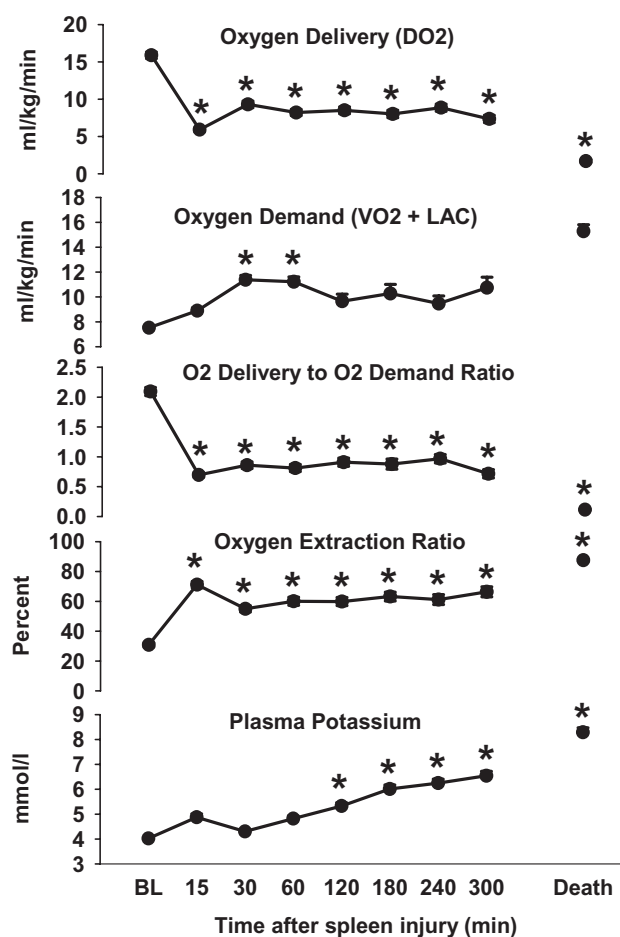

Fig. 8. The time course of the oxygen metabolic responses and plasma potassium levels. There were no differences among the groups, so the data have been pooled. The asterisks denote when the values were significantly different from baseline values.

tated with crystalloid only (data courtesy of LTC R.S. Kotwal, MD, MPH).

Military units operating in isolated, dispersed areas have been known to carry 2 to 4 units of blood products on helicopters, including FFP and RBCs, to administer as an initial resuscitation fluid to treat casualties far forward. Limiting the blood product volume administered in this study to the equivalent of the 4 units that might be carried far forward resulted in similar levels of Hb, plasma protein, fibrinogen, and PLTs; indices of coagulation; and comparable hemodynamic and oxygen metabolic status. These similarities may explain the lack of an effect among

the different plasma : RBCs ratios. The association of a survival benefit of a higher ratio of plasma to RBCs has been demonstrated in patients receiving massive transfusion defined as 10 units or more of RBCs per 24 hours.[26] In those studies that included patients who did not receive massive transfusion, the benefit of the higher plasma-to-RBC ratio has not been found,[27] in agreement with our study.

Hextend resuscitation caused the most bleeding in this model of uncontrolled hemorrhage. It is not possible to discern between a direct effect of Hextend on coagulation and the PLT function or a dilutional effect of Hextend that decreased clotting factor concentrations as potential mechanisms to account for these results. A number of in vitro studies in the literature have found that hetastarch, particularly of the high-molecular-weight and substitution ratios of the formulation used in this study, interferes with coagulation by inhibiting Factor (F)VIII activity and PLT aggregation (coating the PLTs)[28] and interfering with

FXIII-fibrin cross-linking resulting in a weak clot formation.[29] Previous in vivo studies have demonstrated more bleeding with hetastarch resuscitation in animal uncontrolled hemorrhagic models[30] and surgical patients,[31,32] but the dose of colloid was larger than recommended for clinical use.[25] In the present study, the changes in INR, PT, PTT, TEG-K, TEG-angle, TEG-MA, and TEG-G after Hextend infusion at the recommended dose (1000 mL per 70-kg man) are consistent with a measurable reduction in coagulation function. Most of the changes in the coagulation functions after Hextend infusion, while significant from baseline, were within the normal range for all of the variables except PT, PTT, and TEG-MA (Figs. 3 and 4). However, the Hct reduction with Hextend was almost twice that caused by an equal volume of FFP infusion, suggesting that the administration of an identical volume of Hextend resulted in significantly greater volume expansion than FFP. This large-volume expansion effect of high-molecular-weight hetastarch has been observed in previous studies in humans[33] and hemorrhaged dogs,[34] although not in all studies.[35] Thus, the deleterious effects of Hextend on the coagulation status seen in this study could also be related to dilution of the coagulation factors, as has been suggested by a recent study by Cabrales and colleagues.[36] Since Hextend contains calcium, it was exempted by the US FDA to limit its use because of coagulation interference. A study by Deusch and coworkers[37] showed that Hextend caused an increase in the GpIIb-IIIa availability on PLTs compared to other hetastarch formulations that did not have calcium, indicating that Hextend induced less of a direct coagulation defect and lends further support that the dilution effect of Hextend was important in this study.

Another potential explanation for the relatively greater bleeding caused by Hextend and the trend for relatively greater bleeding in the groups receiving RBCs or FWB may be due to the treatments' higher viscosity. The use of a high-viscosity fluid has been recommended to support tissue perfusion and metabolism. Studies from the Intaglietta laboratory[38] suggest that a higher viscosity fluid will increase functional capillary density (FCD) to promote better tissue perfusion. However, infusion of fluid to increase FCD could exacerbate bleeding. For example, the viscosity of Hextend is equal to that of blood (4 cP). Thus, if Hextend and blood increase FCD at the site of the injury, they both could promote bleeding. In contrast, LR and FFP have viscosities of 1 and 1.4 cP, respectively. Although the initial reduction in fibrinogen concentration with LR was equal to that of Hextend, LR's rheologic properties could account for the lower blood loss seen.

There was minimal bleeding after the first 15 minutes after spleen injury with no resuscitation. Resuscitation with any fluid caused higher blood loss but also prolonged survival time because of increased perfusion of vital organs. Previous studies[39-42] have shown the advantages of hypotensive resuscitation strategies in reducing blood loss in the treatment of uncontrolled hemorrhage compared with full resuscitation to a normal blood pressure. In the present study, transfusion of blood products acted as an intravascular hemostatic agent to reduce postinjury hemorrhage volume when administered with hypotensive resuscitation strategies compared with hypotensive resuscitation with Hextend. Although the blood products did not significantly reduce the postinjury blood loss compared with LR treatment, the plasma volume expansion capability of LR was transient as can be seen by time course of the changes in plasma protein concentration (Fig. 5) and the accumulation of fluid in the tissues (Fig. 6).

The addition of oxygen-carrying capacity by infusion of RBCs did not improve survival, suggesting that hypovolemia leading to hypoperfusion and inability to normalize the DO_2-to-demand ratio were major contributors to death in this model. There were uniform responses in hemodynamics, DO_2, acid base balance, metabolic status, and similar reactions of kidney function variables (creatinine, potassium) among the treatment groups. The reason there were no differences in many of the measured variables may be that all of the groups received comparable resuscitation volume treatment. Thus, even a partial restoration of the plasma volume may be the most important lifesaving effect of the initial resuscitation in this otherwise lethal injury[41,43] and that the composition of the blood product resuscitative fluid may not be so important early in the treatment of injury in the maintenance of hemodynamics and metabolism. A corollary may be that with a nonlethal injury, the plasma volume expanders that have the least potential for complications (e.g., immunogenic mismatching) should be the preferred treatment; reserving blood products for situations in which even the smallest advantage can be the difference between life and death in the severely bleeding patient. It should be noted that the swine RBCs were equivalent to 2-week-old human blood based on potassium and lactate concentration accumulation and had no defect in oxygen-carrying capacity as measured by the oxygen dissociation curve (unpublished observations), so the issue of the age of the RBCs is unlikely to be an explanation of the lack of effect in oxygen metabolism among the treatment groups in this model. There was no difference among all the blood components, including FWB. Although this is a model of uncontrolled hemorrhage, it is not a model of massive transfusion where the benefit of FWB or the addition of RBCs could have been demonstrated.

The transient increase in PT seen with LR, along with the trend for increased blood loss, indicates that small changes in this standard coagulation assay correlated with increased bleeding tendencies in our model suggesting that actual PT values may not have to be abnormal before

more bleeding occurs, in agreement with recent findings in humans (K. Brohi, personal communication, 2009). The results of this study demonstrate the importance of practicing damage control resuscitation strategies in the presence of uncontrolled, noncompressible bleeding. While resuscitation increases bleeding in the presence of an injury, limited fluid administration to partially restore plasma volume is necessary to sustain life. In addition, although hypotensive resuscitation minimizes blood loss,[42] the limited volume used in the current study was not sufficient to support 5-hour survival for all animals.[41] DO_2 and the ratio of DO_2 to O_2 demand was equivalent in all groups, and while improved compared with post-shock values, they were not restored to normal levels by fluid resuscitation. The consequences of this could be seen in the gradual increase in creatinine and potassium levels in blood indicating compromise of renal function. There was also a trend for indices of compromised liver (AST) and muscle (creatine kinase) function.

A possible limitation of this study is the use of swine model to assess the hemostatic effect of different resuscitation strategies. Pigs are considered to be hypercoagulable compared to humans and have higher concentrations of many of the clotting factors (FV, FVII, FVIII, F IX, and FXII).[44] Although we have confirmed the higher concentrations of the factors in our pigs (unpublished observations), the pigs' ability to form a clot as measured by the TEG are quite comparable for R, K, and angle variables although the MA can reach higher levels (Table 1). On the other hand, the initial hypercoagulable response of our pigs at 15 minutes to this hemorrhage procedure parallels the finding that most human trauma patients become hypercoagulable in the early phase after injury.[45,46] The issue of the acute coagulopathy in some trauma patients with massive injuries and shock with higher mortality rates[16] was not an issue addressed in this study.

In conclusion, this study demonstrated that the use of blood products as an initial resuscitation fluid reduced blood loss from a noncompressible injury compared with Hextend. When only a few units are necessary or available, FFP was as good an initial resuscitation fluid as those containing RBCs, providing support for the use of plasma as an initial prehospital resuscitation fluid for use in military environments or even civilian situations. There were no differences on PRBL among RBCs-to-FFP, FWB, or FFP in this nonmassive transfusion model.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

ACKNOWLEDGMENTS

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